

ToxCast on Target

In Vitro Assays and Computer Modeling Show Promise for Screening Chemicals

Government agencies rely on toxicity data to help make key regulatory decisions on pesticides and other chemicals in the environment. But thousands of chemicals, including many currently in commerce, have yet to be tested for potential toxicity and impact on human diseases. Such testing can be time-consuming and expensive; a full set of regulatory tests for a single chemical may use thousands of animals and cost millions of dollars. To help address this large backlog of untested chemicals, the U.S. Environmental Protection Agency (EPA) recently completed the first phase of its large-scale ToxCast™ program, which looks at the potential for combining faster and less expensive *in vitro* assays with computer modeling to screen and prioritize chemicals for human toxicity [EHP 118:485–493; Judson et al.]. This approach could both reduce the need for animal testing and speed up the regulatory process.

The ToxCast program evaluates the use of *in vitro* assays for understanding the types of molecular and pathway perturbations caused by chemical exposures and computer-based prioritization models for *in vivo* toxicity. In phase I, researchers chose 309 chemicals for which toxicity data were already available. Using 467 *in vitro* assays across 9 technologies, including high-throughput cell-free assays and

cell-based assays in multiple human primary cells and cell lines, they investigated a broad spectrum of chemical activities at the molecular and pathway levels. Matching these *in vitro* results with existing data helped researchers build initial prioritization models for predicting toxicity of similar but untested chemicals.

This process also provided information on underlying mechanisms of toxicity, which are difficult to investigate directly using animal models. Using human cells or human cell constituents allowed researchers to measure the effects of chemicals on toxicity pathways that may be relevant to human disease. Based on the phase I examples, the ToxCast researchers feel confident that *in vitro* high-throughput data can help predict mechanisms of action for many other well-studied chemicals and indicate which other biological pathways may also be activated. This will lay the groundwork for screening untested chemicals and provide vital guidance for future testing.

The authors hope molecular and computational models will help better guide targeted testing of environmental contaminants but caution that building this new paradigm will itself take time and require input from multiple government organizations. They are launching a second phase of ToxCast to expand on and further confirm that *in vitro* testing can help predict human toxicity. Phase II could be completed over the next several years.

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Attention-Worthy Association

Prenatal Phthalate Exposure and Later Child Behavior

Human exposure to phthalates is ubiquitous due to widespread commercial use. Although the compounds are reported to be rapidly metabolized, concentrations in the body appear to remain fairly stable due to ongoing exposure. The United States and Europe have banned some phthalates from consumer products primarily on the basis of reproductive toxicity data. However, not all phthalates are regulated; meanwhile, research indicates toxicity may extend to other endocrine targets such as the thyroid gland, which is critical for proper neurodevelopment. A new study now reports an association between prenatal exposure to certain phthalates and adverse effects on test scores used to evaluate children's behavior and executive functioning [EHP 118:565–571; Engel et al.].

The prospective study was based on a multi-ethnic cohort of 404 women recruited during their first pregnancy for the Mount Sinai Children's Environmental Health Study between 1998 and 2002. Each woman completed medical, sociodemographic, and lifestyle questionnaires and provided a urine sample between 25 and 40 weeks of pregnancy, which was used to measure phthalate metabolites. Metabolites were grouped according to their molecular weights.

When their children were approximately 4.5–5.5, 6–6.5, and 7–9 years old, 188 of the women completed the Behavior Rating Inventory of Executive Functioning (BRIEF) and the Behaviors Assessment System for Children-Parent Rating Scales (BASC-PRS), standardized forms used in clinical and research assessments of children's executive functioning and behavior. Executive functions encompass planning to achieve goals, controlling attention and emotion,

inhibiting inappropriate behaviors, and extrapolating from life experiences. Problematic behaviors assessed included hyperactivity, aggression, poor conduct, and issues with anxiety, attention, and adaptability.

High-molecular-weight phthalates—like those found in medical tubing and vinyl floor and wall coverings—were not associated with altered scores derived from the parent-report forms aside from a small association with reduced emotional control. However, low-molecular-weight phthalates—like those found in personal care products such as perfume, shampoo, cosmetics, and nail polish—were significantly associated with increased scores for aggression, attention and conduct problems, and depression.

The BASC-PRS includes a scale to help researchers weed out inaccurate assessments. The higher the resulting *F*-score, the more likely the assessment is to reflect an excessively negative evaluation of the child, a failure to follow instructions, random responding, or difficulty reading. When parent-report forms were restricted to those with *F*-scores of 0 or 1 (leaving 161 children in the analyses), most associations remained strong for boys but not girls. The sole exception was conduct problems, which remained significant for both girls and boys.

The behavioral problems assessed in this study are relevant to conditions such as oppositional defiant disorder, conduct disorder, and attention deficit/hyperactivity disorder. Diagnosing these conditions requires extensive testing beyond the scope of this study. Furthermore, this study cannot confirm that phthalate exposure caused these problems via altered thyroid function—or any other mechanism. However, thyroid-related phthalate toxicity makes a connection biologically plausible and underscores an urgent need to further investigate the effects of phthalates on neurodevelopment.



Low-molecular-weight phthalates are found in many personal care products.

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Brain Drain?

PBDEs Alter Development of Human Brain Cells

A new laboratory study demonstrating that polybrominated diphenyl ethers (PBDEs) can alter human fetal brain cells may explain at least in part the neurotoxicity recently documented in epidemiologic studies of young children exposed to PBDEs and previously shown in animal models [*EHP* 118:572–578; Schreiber et al.]. The new study, the first to examine PBDE neurotoxicity in a human cell-based system, links the brain cell alterations to endocrine disruption.

A wealth of data demonstrates that babies can be exposed to significant amounts of PBDE flame retardants both in the womb and through breastfeeding. Although all 3 PBDE formulations—penta, octa, and deca—are banned in Europe, and the penta and octa formulations were discontinued in the United States (deca also is banned in some states), PBDEs may still be used in some new U.S. products and in wares manufactured elsewhere. They also are found in a wide variety of older plastic consumer goods that remain in use in many homes, businesses, and automobiles. The PBDEs are known to migrate into indoor dust, posing a particularly high exposure risk to infants and toddlers because of their characteristic hand-to-mouth behavior.

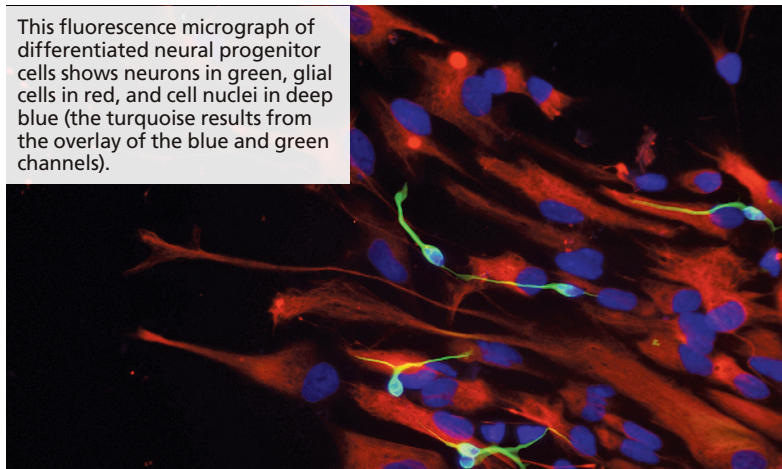
To investigate how PBDEs may impact the developing fetal brain, the team of scientists employed a method for evaluating human developmental neurotoxicity they had recently developed [*EHP* 117:1131–1138] as an alternative to animal testing. This method uses primary fetal human neural progenitor cells (hNPCs) cultured to produce complex 3-dimensional cellular systems called neurospheres. Neurospheres undergo the same basic processes that occur during the early stages of normal human brain development: cell proliferation, differentiation, and migration. Tests conducted with neurospheres may help identify exogenous substances that disturb these basic processes *in vivo*.

The researchers focused on 2 of the PBDE compounds that accumulate the most in humans, BDE-47 and BDE-99. At concentrations below levels that cause cell death, they found these compounds could reduce the migration of the hNPCs—which suggests the possibility of adverse effects on brain development—and the effects increased with higher PBDE concentrations. At the highest tested concentration (10 μ M), BDE-47 decreased the distance the cells migrated by more than 25% compared with unexposed cells, whereas the same

concentration of BDE-99 decreased the distance by more than 30%. Additional testing established that both compounds also interfered with the differentiation of immature progenitor cells into neurons and oligodendrocytes.

Further tests suggested the PBDE compounds affected cell migration and differentiation by interfering with thyroid hormone signaling, an endocrine-disrupting effect that could be associated with additional impacts throughout a person's life. Followup work to determine whether PBDEs cause the same effects in rodent neurospheres would facilitate extrapolation from animals to humans, the authors say.

This fluorescence micrograph of differentiated neural progenitor cells shows neurons in green, glial cells in red, and cell nuclei in deep blue (the turquoise results from the overlay of the blue and green channels).



Other work has shown the tendency of PBDEs to accumulate in brain and neuronal cells, and the researchers used radiolabeled BDE-47 to measure an accumulation in test cells of approximately 60-fold. Considered with available human exposure data, these data suggest current PBDE exposure levels are likely to be of concern for human health, the authors write. Two recent studies have linked PBDEs with subtle changes in children's IQs and behavior [*EHP* 117:1953–1958 and *EHP* doi:10.1289/ehp.0901340], and the authors say additional studies are needed to assess these associations.

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Chew on This

Persistent Organic Pollutants May Promote Insulin Resistance Syndrome

Animal studies indicate some persistent organic pollutants (POPs) may be endocrine disruptors and suggest similar health risks for humans. Recent studies have further suggested an association between exposure to POPs and the prevalence of type 2 diabetes. Now an experimental animal study reports evidence of a causal link between POP exposure and insulin resistance syndrome, a cluster of metabolic disorders—including type 2 diabetes—that are marked by sustained high blood sugar [*EHP* 118:465–471; Ruzzin et al.].

POPs accumulate in fatty tissue, where they can remain for years because they are not easily broken down. Fatty fish are a potential source of POP exposure in many human populations. However, *n*-3 polyunsaturated fatty acids in fish oil may have beneficial health effects, possibly including protective effects on insulin resistance, that could counterbalance any adverse effects of POPs in fatty fish.

In the current study, rats were exposed for 28 days to high-fat diets that contained either crude fish oil (from farmed Atlantic salmon) or fish oil that was refined to remove POPs. As expected,

the crude fish oil contained much higher levels of POPs than the refined oil. Gene expression profile comparisons of the livers of the 2 treatment groups showed that POP exposure disrupted lipid homeostasis.

Rats on the unrefined fish oil diet gained more weight overall and had increased triacylglycerol, diacylglycerol, and total cholesterol levels compared with rats fed refined fish oil. They also showed impaired insulin action in response to the high-fat diet, whereas the high-fat diet did not seem to cause insulin resistance in the rats fed refined fish oil. Further analysis revealed a reduction in the ability of insulin to stimulate glucose uptake in adipocytes treated with a mixture of POPs that was comparable to the mixture of chemicals in crude fish oil. Adipocyte responses to insulin varied with exposures to different mixtures of individual POPs.

The authors conclude that dietary exposure to POPs may be a risk factor for insulin resistance and associated metabolic disorders. Furthermore, the metabolic effects of POP exposures exacerbated deleterious effects of a high-fat diet on rats and appeared to negate protective effects of *n*-3 polyunsaturated fatty acids on insulin resistance.

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